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2017-01

Chen , Z , Setala , H , Geng , S , Han , S , Wang , S , Dai , G & Zhang , J 2017 , ' Nitrogen addition impacts on the emissions of greenhouse gases depending on the forest type : a case study in Changbai Mountain, Northeast China ' , Journal of Soils and Sediments , vol. 17 , no. 1 , pp. 23-34 . https://doi.org/10.1007/s11368-016-1481-7

http://hdl.handle.net/10138/308224
https://doi.org/10.1007/s11368-016-1481-7

unspecified
acceptedVersion

SOILS, SEC # • RESEARCH ARTICLE

Nitrogen addition impacts on the emissions of greenhouse gases depending on the forest type: a case study in Changbai Mountain, Northeast China

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Abstract

Purpose Anthropogenic-induced greenhouse gas (GHG) emission rates derived from the soil are influenced by long-term nitrogen (N) deposition and N fertilization. However, our understanding of the interplay between increased N load and GHG emissions among soil aggregates is incomplete.

Materials and methods Here, we conducted an incubation experiment to explore the effects of soil aggregate size and N addition on GHG emissions. The soil aggregate samples (0-10 cm) were collected from two six-year N addition experiment sites with different vegetation types (mixed Korean pine forest vs. broad-leaved forest) in Northeast China. Carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) production was quantified from the soil samples in the laboratory using gas chromatography with 24h-intervals during the incubation (at 20°C for 168 h with 80% field water capacity).

Results and discussion The results showed that the GHG emission/uptake rates were significantly higher in the micro-aggregates than in the macro-aggregates due to the higher concentration of soil bio-chemical properties (DOC, MBC, NO_3^-, NH_4^+, SOC and TN) in smaller aggregates. For the N addition treatments, the emission/uptake rates of GHG decreased after N addition across aggregate sizes especially in the mixed Korean pine forest (PF) where CO_2 emission was decreased about 30%. The significant effects of N addition on GHG emissions on SOM basis were recorded in the PF rather than in the broad-leaved forest (SF) suggested that N addition decreased of GHG emission/uptake rates through change in the quality of SOM in the PF forest. The global warming potential (GWP) which was mainly contributed by CO_2 emission (>98%) was decreased in the PF after N addition but no changes in the SF.

Conclusions These findings suggest that soil aggregate size is an important factor controlling GHG emissions through mediating the content of substrate resources in temperate forest ecosystems. The inhibitory effect of N addition on the GHG emission/uptake rates depends on the forest type.

Keywords Greenhouse gas • N deposition • Soil aggregate • Soil organic carbon

1 Introduction

Anthropogenic-induced greenhouse gas (GHG) emissions produced from soil to the atmosphere have increased steadily since the industrial revolution . Carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) are the main components of GHGs, which accounts for more than 80% of global warming . The increasing concentrations of GHGs are set to continue in many industrialized regions, including the eastern regions of China . Of the total anthropogenic emissions (8.6 Gt C yr^{-1}), only half are absorbed by the ocean or by unidentified terrestrial sinks . The mitigation of GHG emissions from the terrestrial ecosystem requires the reduction of direct emissions from sources through the enrichment of carbon (C) and nitrogen (N) sinks in the soil . Temperate forest ecosystems sustain approximately 20% of the world's plant biomass and 10% of terrestrial C. In addition, temperate forests have the potential to store and sequester C and therefore, mitigate global warming; however, they can be influenced by various factors, including biotic (e.g. anthropogenic activity) and abiotic (e.g. N deposition) factors .

The increased concentration of GHGs can in turn impact the decomposition of soil organic matter (SOM) and carbon storage. The increased concentration of N_2O in the atmosphere has doubled the rate of N inputs into most terrestrial ecosystems . The effect of N deposition on SOM decomposition and C storage has received significant attention in recent years. For example, the increased N deposition due to the increased concentration of N_2O in the atmosphere has been found to increase , decrease or have no effect on the soil C content. In addition, the increased N deposition is reported to promote soil GHG emissions/uptakes due to changes in soil N status and microbial communities . This effect could increase GHGs in the atmosphere and subsequently aggravate global warming. However, there is still a significant knowledge gap in our understanding of the mechanisms by which N deposition directly impacts GHG releases and C storage through changes in the chemical and biological properties of soil . Studies on the effects of soil structure or soil aggregate distribution on GHG emissions due to N deposition are rare; however, these studies are urgently needed to facilitate decision-making in forest management. Diba et al. (2011) reported that N_2O

emissions could be significantly influenced by soil aggregate size and N addition due to differences in soil properties in various aggregate sizes.

The physical protection of SOM through the formation of organo-mineral associations within soil aggregates is one of the most crucial but poorly understood stabilization mechanisms of SOM in soil . Soil aggregates physically protect the SOM from various decomposing agents and then stabilize the SOM through several mechanisms, including through separated enzymes and substrates on mineral and humic surfaces , suppressed microbial activity due to inhibited access to gases or nutrients , and separated decomposer organisms to SOM within small porous systems . These mechanisms suggest that the locations of SOM and the soil nutrient properties in aggregates control the rate of SOM decomposition and therefore, control the emission rates of GHGs. The emission rates of GHGs from SOM decomposition differ significantly among aggregate sizes (e.g. macro-aggregates vs. micro-aggregates; Six and Paustian (2014)). Tao and Song (2013) found that C mineralization was higher in micro-aggregates than in macro-aggregates in clay loam soil. However, Elliott et al. (1996) observed a contrasting trend and found that the rate of C and N mineralization was greater in macro-aggregates than in micro-aggregates. Mangalassery et al. (2013) reported that the CO_2, N_2O and CH_4 emission/uptake rates were greater in the micro-aggregates than in the macro-aggregates with clay loam texture; however, the emission/uptake rates were greater in macro-aggregates than in micro-aggregates with sandy loam texture. Therefore, although SOM in macro-aggregates was more labile and less processed than that in micro-aggregates, the concentration of SOM in micro-aggregates under clay-enriched soil was much higher than in macro-aggregates and subsequently resulted in higher emission rates of GHGs. However, Aoyama et al. (1999) reported that crushing macro-aggregates enhanced C mineralization by 14-35%. Similar observations were also made by Mueller et al. (2012), who reported that the dispersion of aggregates using ultrasonic treatment resulted in an increase in C mineralization by 27% due to an increase in labile organic carbon. Therefore, the emission rates of GHGs in soil aggregates can be controlled not only by soil properties but also by aggregate stability.

The majority of recent studies have focused on the effects of land use and management on soil aggregation and GHG emissions, but few studies have determined the effect of N deposition on soil aggregation and the emission rates of GHGs in aggregates. However, Plaza-Bonilla et al. (2014) observed that inorganic N fertilization promotes the emission of CO_2 and N_2O in an incubation experiment using agricultural soil. Diba et al. (2011) detected that the impact of N addition on N_2O emission depended on soil aggregate size in grassland soil. Tao and Song (2013) reported that N addition had a neutral and negative effect on SOC decomposition in wetland soil. It is generally believed that the patterns of GHG emissions are less evaluated in soil aggregates in forest ecosystems that incur significant N deposition, especially in Northeast China (> 23 kg N yr^{-1} ha^{-1}).

Therefore, a six-year *in situ* N addition experiment in mixed Korean pine forest and broad-leaved forest was established at Changbai Mountain in Jilin Province, northeastern China. We hypothesized the following: (1) N addition would induce positive effects on GHG emissions in the temperate forest soil; (2) compared with the mixed Korean pine forest, the broad-leaved forest would provide resources richer in nutrients, which would translate into increased GHG emissions, and (3) the small-sized aggregates with a larger surface area would capture more nutrients than the larger aggregates and therefore, would produce higher GHG emissions. To test these hypotheses, first, the concentration of C and N, and other properties of the aggregates were determined from two N addition experiment sites with different vegetation composition. Second, the GHG emission rates from the soil aggregates were measured in a laboratory incubation experiment.

2 Materials and methods

2.1 Site location

The study sites were located in the research forest of the Changbai Forest Ecosystem Research Station (CBFERS; established in 1979) in Northeast China. A secondary forest (SF) and a primary forest (PF) were selected for the study. The SF is a 30-year-old broad-leaved forest (42.517 N, 127.783 E, Jilin Province, China) and is dominated by birch (*Betula*

platyphylla Suk) and aspen (*Populus davidiana*) trees. The mean annual precipitation and temperature at this site are 871.6 mm and 2.7ºC, respectively. The PF is a mixed Korean pine old growth forest and is close to the city of Erdaobaihe (42.402N, 128.096E). The mean annual precipitation and temperature at this site are 700mm and 3.8 ºC. The dominant plant species in the PF are *Quercus mongolica* and *Pinus koraiensis.* The soil at both study sites is classified as Mollisols after the American Soil Taxonomy with high organic matter content in the surface soil layer. The ambient N deposition, measured from throughfall at the study sites, is 23 kg N ha^{-1} yr^{-1} . A more detailed description of the study sites is presented in Table 1.

2.2 Experimental design

Two levels of N addition (0 kg N ha^{-1} yr^{-1} (N0); 50 kg N ha^{-1} yr^{-1} (N50)) with three replications were randomly arranged into 6 blocks at both sites (30×30 m for each block in SF and 25×50 m for PF). Ammonia nitrate (NH_4NO_3) aqueous solution (40 L) was applied to the treated plots monthly during the growing season (4-10 months) each year starting in 2007 (SF) and 2009 (PF). Four soil samples from each block were collected to make one composite sample from the A-horizon (0-10 cm) in October 2012 (SF) and May 2014 (PF). The soil samples were brought into the laboratory where plant litter, fine roots, and larger soil fauna were removed. The remaining soil was then gently sieved using an 8-mm sieve and air-dried at room temperature. The soil was then separated into five sizes of aggregates and stored at 4ºC before incubation.

2.3 Aggregate preparation

The air-dried soil samples were passed through a series of sieves (4 mm, 2 mm, 1 mm, 0.25 mm and 0.053 mm) using the wet sieve method to obtain five aggregate size-fractions of 4-8 mm, 2-4 mm, 1-2 mm, 0.25-1 mm, and 0.053-0.25 mm (free micro-aggregates, free-m). Briefly, the air-dried soil (100 g) was moistened in distilled water for 10 min and then shaken in a container with distilled water for 10 min. The separated aggregates were oven-dried at 60° C, and then weighed. The field water capacity (FWC) was measured using a funnel

according to the methods of Diba et al. (2011), and the values of the FWC from the largest aggregates to the smallest aggregates were 1.01 g g^{-1}, 1.03 g g^{-1}, 1.18 g g^{-1}, 1.59 g g^{-1}, 1.99 g g^{-1}, respectively.

2.4 Incubation procedure

The soil aggregate samples (10 g each, dry) were loosely packed into 300 ml incubation flasks. All samples for each aggregate size (with 3 empty flasks as controls) were adjusted to 80% FWC moisture and then balanced for 2 h before gas sampling. After the first sampling, all samples were incubated at 20°C for 168 h. The flask necks were sealed by a semi-permeable membrane to prevent the samples from drying. The gas samples from the dry soil (before incubation) and wet soil (after soil wetting) were taken using a 100 ml syringe and then analyzed by gas chromatography (Agilent 7890A, USA). The concentrations of CO_2 and CH_4 were detected using an infrared ion detector and the concentration of N_2O was detected using an electron capture detector. The gas samples were taken at regular intervals (2 h, 24 h, 48 h, 72 h, 120 h, and 168 h). The levels of NH_4^+-N, NO_3^--N, dissolved organic carbon (DOC), and microbial biomass carbon (MBC) of the aggregate soils were measured before and after incubation, and the total carbon (TC) and total nitrogen (TN) levels were measured before incubation.

We performed sand-corrections to analyze the concentration of C and N in the aggregate fractions using the following formula (Eq. (1)):

$$\text{Sand free } C_{fraction} = \frac{C_{fraction}}{1 - sand_{fraction}} \quad (1)$$

where $C_{fraction}$ is the C concentration (g kg^{-1}) in the different aggregate sizes and $sand_{fraction}$ is the fraction of sand in that aggregate size. To calculate the sand free N in the aggregate fraction, the concentration of N in the fraction was used instead of the C concentration in Eq. (1).

The gas production rate (F, mg kg^{-1} h^{-1} or μg kg^{-1} h^{-1}) was calculated according to the methods of Diba et al. (2011) using the following equation (Eq. (2)):

$$F = \rho \times \frac{V}{W} \times \frac{\Delta c}{\Delta t} \times \frac{273}{T} \quad (2)$$

where F is the gas emission rate (mg kg^{-1} Agg h^{-1}) of the aggregates, ρ is the density of gas (μg m^{-3}) at 273 K and 1 atm., V is the headspace volume of the flask (m^3), W is the dry soil weight (kg), △c is the gas concentration change in the flask (m^3 m^{-3}), △t is the incubation period (h), and T is the air temperature (K) in the flask.

The cumulative gas emission during the incubation was calculated using the following equation (Eq. (3)):

$$\text{Cumulative } F = \sum(Fi \times Ti) \quad (3)$$

where Fi is the mean gas production rate of the sampling intervals (mg kg^{-1} h^{-1}) and Ti is number of hours in the sampling intervals.

2.5 Contribution of SOM to GHG emissions from aggregate fractions

To determine the interaction effect of SOM and N addition on GHG emissions, the GHG emissions from each aggregate fraction were calculated on per SOM basis using the following equation (Eq. (4)):

$$\text{The GHG emission rate from SOM (mg } kg^{-1} \text{ SOM } h^{-1}) = F \text{ (mg } kg^{-1} \text{ Agg } h^{-1}) / \text{SOM concentration in the aggregates (g } kg^{-1} \text{ Agg)} \times 10^3 \quad (4)$$

2.6 Estimation of global warming potential

The net global warming potential (GWP, mg CO_{2e} GHG emission kg^{-1} Agg) was estimated using the radiative force in a time horizon of 100 years of the different greenhouse gas molecules . For computing the GWP, the GHG emission rate was expressed as a CO_2 equivalent (CO_{2e}) using Eq. (5):

$$GWP = CO_2 \text{ (g } kg^{-1} \text{ Agg)} + 298 \times N_2O \text{ (g } kg^{-1} \text{ Agg)} + 25 \times CH_4 \text{ (g } kg^{-1} \text{ Agg)} \quad (5)$$

where CO_2, N_2O and CH_4 represent the 7-day cumulative emissions of the individual GHGs.

2.7 Statistical analysis

Statistical analysis was performed using SPSS 13.0 (SPSS Inc., USA). The effects of the two factors (aggregate size (S) and N addition (N)) and their interactions on gas production and the soil properties in each forest site were analyzed using a general linear model ANOVA. Natural-log transformations were applied when it is necessary to meet the assumptions of ANOVA. To determine the effects of soil initial properties on GHG emissions, the correlation between GHG emissions and soil properties before incubation was analyzed using a two-tailed Pearson's correlation at a significance level of 0.05.

3 Results

3.1 Soil properties in aggregates

The bio-chemical properties (NH_4^+, NO_3^-, DOC and MBC) of the soil aggregates were measured before and after incubation, and SOC and TN were measured before incubation (Table 2). After incubation, the levels of NH_4^+ and NO_3^- increased in the SF, while the opposite trend was observed in the PF. In addition, DOC increased and MBC decreased after incubation at both the SF and the PF. In the aggregate size classes, all bio-chemical properties were higher in the free-m, and lower in the macro-aggregates at both forest types. The influence of N addition on the aggregate bio-chemical properties was not significant with the exception of DOC before incubation at both forest sites. The concentration of DOC in aggregates decreased after N addition in the SF, but increased in the PF.

3.2 Aggregate gas release

At both forest sites, 7-day cumulative CO_2 emissions increased substantially during the first seven days after the start of incubation. The CO_2 emissions in the PF were significantly affected by aggregate size and N addition ($P<0.01$, Table 3), while only affected by aggregate size significantly in the SF. Cumulative CO_2 emissions increased as the aggregate size decreased at both forest sites. At both forest sites, cumulative CO_2 emissions from the soil aggregates were reduced after N addition, especially in the PF soil where CO_2 production was reduced by approximately 30% compared with N0.

The 7-days cumulative N_2O emissions were significant affected only by aggregate size (P<0.01, Table 3). N_2O emissions increased rapidly during the first 24 h after the start of incubation (Figs. 1 and 2). On average, the 7-days cumulative N_2O emissions under fertilized plots (9.36 μg kg^{-1} Agg) were similar to control plots (8.97 μg kg^{-1} Agg) in the SF, while slightly decreased (7.26 vs. 5.31 μg kg^{-1} Agg for N0 vs. N50) after N addition in the PF across aggregate sizes. The 7-day cumulative N_2O emissions were lower in the medium-sized aggregates (2-0.25 mm) compared with the 4-8 mm and free-m aggregates for the N addition treatments at both forest sites.

Regarding the aggregate size, the cumulative CH_4 uptake was higher in the free-m aggregates compared with that in the macro-aggregates at both forest sites. Furthermore, the cumulative CH_4 uptake remained unchanged in the macro-aggregates in the PF, but increased as the macro-aggregate size decreased in the SF. The treatments of N addition showed no effect on the CH_4 uptake across all aggregate sizes (P>0.05, Table 3), although decreased CH_4 uptake in the PF (~20%) and increased CH_4 uptake in the SF (~15%) were observed after N addition.

3.3 Gas fluxes expressed in terms of SOM

When the GHG flux data were expressed in terms of organic matter, the differences in all three gas flux patterns between aggregate sizes and N treatments were similar to the per soil patterns (Table 4). Briefly, higher CO_2 emission rates were recorded in the smaller aggregate sizes across the N addition groups. Compared with control, the emission rates of CO_2 were lower under fertilized samples at both forest sites. The patterns of the average CH_4 uptake rates expressed per SOM were higher in smaller aggregates in the SF site, while lower CH4 uptake rates were recorded at medium-size aggregates (0.25-2 mm) in the PF. The average CH_4 uptake rates were inhibited after N addition across aggregate sizes.

In all treatments, the average N_2O emission rates per SOM were similar to the average N_2O emission per soil across aggregate sizes and N addition (Table 4). However, the average N_2O emission rates per SOM after N addition treatment were significantly lower than the

control plots, especially in the PF soil (P =0.024).

3.4 Global warming potential

The CO_2 emissions were the greatest contributor to the GWP (Fig. 3). It was identified that CH_4 contributed negatively to the GWP at both forest sites because of the net CH_4 uptake by the soil during incubation. The GWP from the SF was significantly higher (148.3%) than that of the PF soil. For N treatment, there was a significant difference in the GWP between N0 and N50 in the PF, but no significant effect was found in the SF. An increase trend of GWP was observed at both sites as the aggregate size decreased.

3.5 Relationships between gas emissions and soil properties

Pearson's correlation coefficients among GHG emissions and soil properties of before incubation in each forest site were showed in Table 5. The relationships between soil initial properties and GHG emissions differed between forest sites. In the SF, CO_2 emissions were significant correlated to most of soil properties except for NO_3^- (r=0.180, P>0.05), while in the PF only MBC, SOC and TN were significant correlated with CO_2 emissions (r=0.440, 0.502, 0.550 respectively). The significant relationships between N_2O emissions and soil properties were found only between DOC and N_2O emissions (r=0.445), and NO_3^- (r=0.479) and N_2O emissions in the PF, but all of the soil properties were significantly correlated with N_2O emissions despite of the MBC which was marginally significant (r=0.323, P=0.087). In the PF, only MBC was marginally significant correlated with CH_4 uptake (r=0.327, P=0.077), but in the SF, only MBC and TN did not have any relationship with CH_4 uptake. These relationships differed between forest types indicated that soil nutrients in the SF were easier for microbial utilization than in the PF.

4 Discussion

The objective of this study was to investigate the effects of soil aggregate and N addition on GHG emissions in two different forest sites. Many factors, including the soil biotic and

abiotic properties of different sized aggregates, independently and synergistically influence the emissions of GHG . The differences in soil properties observed before and after incubation were statistically significant (Table 2). The decreased content of MBC after incubation may be the main reason for the decrease in GHG emissions. The increased content of DOC may result from the decreased stability of aggregates from the reciprocated force. The increased content of NH_4^+ and NO_3^- after incubation is similar to results reported by Diba et al. (2011) and can be attributed to N mineralization during incubation. In the present study, we found that N addition and aggregate size influenced GHG emissions with the evidences of changes in various soil properties (Table 2). The details were discussed below.

4.1 CO_2 emission

The CO_2 emissions and the bio-chemical properties of the soil increased as the aggregate size decreased across the different N additions levels at both forest sites (Figs. 1 and 2, Tables 2 and 4). These results were in agreement with Kimura et al. (2012), who suggested that smaller micro-aggregates contained more easily decomposed organic matter with improved availability for microorganisms. However, macro-aggregates were known to contain more labile organic matter as bonding agents between the micro-aggregates , while C enclosed in micro-aggregates was more stable due to a strong combination with soil particles . These differences in the bio-chemical properties of aggregates were supported by the results from Elliott et al. (1996), who found higher CO_2 emissions from macro-aggregates than micro-aggregates. Mangalassery et al. (2013) also found similar trends in sandy loam texture soil, but the opposite effects in clay loam soil. The above studies suggested that the difference in CO_2 emissions of the different aggregate sizes was attributed to the various nutrient contents in aggregates regardless of soil texture. Therefore, our results found higher CO_2 emissions in smaller aggregates because of higher nutrient contents than larger aggregates (Table 2) and suggesting that the soil properties could be a key factor in influencing C mineralization in forest soils.

Nitrogen addition has been reported to increase , decrease and have no effect on CO_2

emissions from soil due to different control factors (e.g., roots or soil organisms). Examining soil from the same area as was used in our study, Wu et al. (2015) also found that both NH_4Cl and KNO_3 addition significantly decreased the CO_2 emissions by 9.2-21.6%, and they attributed this decrease to decreases in C utilization efficiency by microorganisms in the soil. Similarly, in the present study a decreased trend in CO_2 emissions was observed after N addition at both forest sites across all aggregate sizes, but this trend was statistically significant only in the PF soil (~30%). This trend was associated with the decreased of microbial biomass after N addition (Table 2). Similarly Tao and Song (2013) also reported a reduction of C mineralization under N addition due to suppressing microbial biomass. These results suggest that N addition can influence the sink potential in the mixed Korean pine forest.

4.2 CH_4 uptake

CH_4 flux from the soil is dependent on the redox conditions, and soils under anaerobic conditions promote CH_4 formation; however, under aerobic conditions, CH_4 oxidation is favored . In the present study, soil aggregates at both forest sites acted as a sink for CH_4 during incubation except for the initial days (Figs. 1 and 2). This result suggested that the conditions of all the samples during incubation were favored by aerobic CH_4 oxidation.

In present study, the effect of aggregate size on CH_4 emissions differed with forest types. In the SF, CH_4 uptake was higher in smaller aggregates, but the pattern in the PF was less clear. The higher oxidation of CH_4 in the smaller aggregates may result from aerobic conditions and higher resource/nutrient content for microbe metabolism in the SF with the evidence of the relationship between CH_4 oxidation and most of soil properties (DOC, SOC, NH_4^+ and NO_3^-). However, less obvious correlation was found between CH_4 oxidation and soil properties in the PF (only MBC was marginally significantly correlated to CH_4 oxidation, P=0.077), indicating that soil properties did not directly influence CH_4 oxidation in the PF. The small aggregates should confer increased CH_4 emissions because of the reduction in ventilation (boosting anaerobic conditions) in repacked small soil aggregates . Furthermore,

some studies also suggested that anaerobic conditions can be found in the larger macro-aggregates, when fine pore was filled by water after wetting procedure . These characteristic of the soil aggregates may the reasons for the less difference of CH_4 oxidation between aggregate sizes in the PF.

Evidence suggests that soil N status is one of the major factors that influence CH_4 oxidation (see reviewed by Shrestha et al. (2015)). In our study, soil N, including $NH_4^+$and NO_3^-, was significantly and negatively correlated to the CH_4 uptake rates (Table 5), indicating that higher N content limits CH_4 oxidation. Although the results were not significant, the inhibitory effect of N addition on CH_4 uptake was observed only in the PF across all aggregate sizes (Figs. 1 and 2). However, in the SF, CH_4 uptake rate on a per SOM basis in the control plots was similar to the N addition plots, suggesting that higher microorganism activity in the broad-leaved forest soil (high content of MBC) suppresses the effect of N addition on CH_4 oxidation. These results were in agreement with studies that have shown N addition decreased CH_4 oxidation through inhibiting the enzyme activity of CH_4 mono-oxygenase . However, some studies have shown positive effects of N addition on soil CH_4 oxidation , implying that N may be an essential nutrient for methanotrophs to assimilate CH_4 (Table 5; . At the same site, Li et al. (2013) also reported a slight inhibitory effect of N addition on the CH_4 oxidation of bulk soil. Furthermore, a similar incubation study was conducted by Plaza-Bonilla et al. (2014), who also found a slight decrease in CH_4 oxidation in intact macro-aggregates under fertilized treatment compared to control. These results suggest that the addition of N may have negative effect on CH_4 oxidation under mixed Korean pine forest rather than broad-leaved forest.

4.3 N_2O emission

N_2O emissions have been reported to significantly correlate to soil moisture status and quickly reach the maximum emission rate after soil moistening . This initial flush is thought to be a result of a rapid change from aerobic to anaerobic conditions and the concomitant rapid change in microbial activity after soil moistening. In the present study, increasing N_2O

emissions were observed with decreasing aggregate size across the two forest sites, with the exception of the largest aggregates where cumulative N_2O emissions were higher than in other macro-aggregates (Figs. 1 and 2). This agrees with the findings by Bandyopadhyay and Lal (2014) and Plaza-Bonilla et al. (2014), who attributed the higher emissions of N_2O in larger macro-aggregates to the presence of anaerobic microsites within the macro-aggregates, which also exist in the micro-aggregates. The highest cumulative N_2O emissions in this study were recorded in the micro-aggregates with the highest nutrient content (e.g. NH_4^+, NO_3^-) at both forest sites (Table 2).

The effects of N addition on soil N_2O emissions were mostly positive for the field observations . Additionally, similar results were obtained from the same sites used in this study by Bai et al. (2014), who reported a positive effect of laboratory N addition on N_2O emission. Few studies have failed to observe a significant increase in N_2O emissions after N addition , which was attributed to competition for N by trees. Furthermore, there has been limited investigation in the effects of long-term N addition on N_2O emission in aggregates. In our experiment, no difference in N_2O emissions was recorded between N addition treatments in the SF, while a negative effect was found in the PF, especially scaling down to per SOM basis (P=0.024). The N_2O emissions in the SF was correlated with most of soil properties determined in this study, while they only significant correlated with DOC and NO_3^- in the PF (Table 5). These results indicated that soil properties in the SF was easier for microbial utilization than in the PF, and therefore, resulting in different responses of N_2O emissions to N addition.

4.4 GWP

To compare the total effect of N addition on GHG emissions of the two forest sites, the radiative force from the net GHG emissions was calculated using the GWP (Fig. 3). The CO_2 emissions were the greatest contributor to the GWP (> 90%), while CH_4 negatively contributed to the GWP. This finding was in agreement with Bandyopadhyay and Lal (2014) and Shrestha et al. (2014) who also found that CO_2 was the largest contributor to the GWP

(98.9% in forest soil). However, Mosier et al. (2005) found that N_2O emissions accounted for 40-44% of GWP in an agricultural soil, whereas it only accounted for 1% in present study. The different patterns of GWP between agricultural soil and forest soil can be attributed to agricultural production practices (e.g. tillage, irrigation or fertilization etc.) . For N addition treatments, the decreased trend of GWP after N addition mainly resulted from the reduction of CO_2 emissions after N addition due to the highest contributions of CO_2 to GWP (Fig. 3). But several studies including agricultural soil and forest soil reported a positive effect of N addition on GWP in a field investigations. Whereas, no effects of N addition on GWP were also reported Jiang et al. (2010). These different effects of N addition on GWP can be attributed to the differences in soils (agricultural soils or forest soils), N addition treatment durations (short-term or long-term), and fertilizer types (inorganic N or organic N). Therefore, the result in our study suggested that N addition may decrease the GHG emissions and subsequently increase C sequestration in the soil, particularly in mixed Korean pine forests.

5 Conclusions

The GHG emissions from the temperate forest soil were influenced by several factors. Aggregate size and N addition had a significant influence on releases of GHGs from soils. The emission/uptake rates of GHGs in the micro-aggregates were higher than that in the macro-aggregates at both forest sites due to contained more resources in micro-aggregates than macro-aggregates. The addition of N to the soil significantly inhibited the GHG emission/uptake rates in the mixed Korean pine forest soil (PF), while the effect was minimal in the broad-leaved forest (SF). These results indicated that aggregate size and N addition have profound implications on GHG emissions but differed in forest types. The GWP was increased with aggregate size decreased at both forest sites. The effect of N addition on GWP was negative in the mixed Korean pine forest soil, but less clear in the broad-leaved forest soil. These results suggested that N addition decreased the emissions of GHGs depending on the forest type and the various aggregates, and therefore, may increase C sequestration in the mixed Korean pine forest.

Acknowledgements This work was funded by grants from the strategic priority research program of Chinese academy of sciences (XDA05020300), the national natural science Foundation of China (41330530, 41430639, 31470522).

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599

600**Tables**

601

602**Table 1** Soil characteristics at the two forest sites (SF = secondary forest; PF = primary forest)

	pH	SOC (%)	TN (%)	C/N	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	soil texture (%)		
							sand	silt	clay
SF	5.20	22.54	1.71	13.10	21.37	47.10	26	40	34
PF	5.16	15.60	1.03	15.01	31.39	27.18	10	51	38

603Data (Mean) were determined from the bulk soil derived from Lou (unpublished data, 2013).

Table 2 Soil bio-chemical properties under different N addition treatments (N0 vs. N50) and aggregate size classes (mm) at the two forest sites (SF = secondary forest; PF = primary forest)

	NH_4^+ (mg kg^{-1})		NO_3^- (mg kg^{-1})		DOC (mg kg^{-1})		MBC (mg kg^{-1})		SOC (g kg^{-1})	TN (g kg^{-1})
treatment	before	after	before	after	before	after	before	after	before	before
SF-N0										
4-8 mm	112.90	339.12	6.19	25.10	827.28	1516.11	2256.74	1598.74	124.55	11.00
2-4 mm	161.75	329.73	9.23	17.10	814.11	1643.90	2075.55	1273.13	124.78	10.56
1-2 mm	161.31	328.73	12.96	17.64	951.96	1966.08	2321.51	1660.46	116.50	10.02
0.25-1 mm	142.40	342.81	12.63	19.84	1059.53	2036.52	2176.95	1526.58	128.18	10.92
free-m	196.15	405.88	25.10	34.38	1253.49	2686.02	2421.63	2069.65	130.96	11.72
SF-N50										
4-8 mm	94.00	322.10	13.37	17.93	756.87	1633.23	2241.28	1152.03	126.02	10.86
2-4 mm	109.90	332.91	11.74	18.54	655.02	1369.56	1988.18	1355.54	127.77	10.80
1-2 mm	131.70	360.46	12.25	16.06	666.09	1463.38	2250.70	1541.36	128.87	10.62
0.25-1 mm	144.93	373.67	14.27	16.65	793.44	1624.03	2446.20	1472.51	137.80	11.34
free-m	179.42	385.05	27.97	50.84	1129.12	2673.17	3765.61	1951.86	139.89	10.86
P-value of ANOVA of each soil properties in SF										
S	0.240	**0.009**	**0.000**	**0.002**	**0.037**	**0.000**	0.111	0.523	0.354	0.666
N	0.310	0.626	0.101	0.762	**0.067**	0.131	0.230	0.616	0.621	0.915
S × N	0.954	0.432	0.622	0.386	0.856	0.584	0.338	0.974	0.940	0.902
PF-N0										
4-8 mm	305.74	280.52	106.90	127.94	739.25	1633.28	1618.70	1633.28	104.67	7.70
2-4 mm	377.34	281.92	117.28	140.11	709.47	1575.05	1860.45	1575.05	135.37	9.30
1-2 mm	379.71	299.27	130.92	137.33	859.70	1462.74	3028.75	1462.74	150.50	10.07
0.25-1 mm	389.09	309.81	131.74	131.57	933.50	1770.56	3301.67	1770.56	154.43	10.47
free-m	439.05	392.29	219.97	173.04	1342.79	2731.13	3755.86	2731.13	176.13	11.70
PF-N50										
4-8 mm	226.19	264.16	164.96	98.29	811.95	1415.20	2158.27	1415.20	105.77	7.57
2-4 mm	326.32	278.43	133.45	88.52	858.36	1286.60	2210.38	1286.60	131.50	8.73
1-2 mm	369.24	285.87	155.48	108.31	922.91	1378.44	2070.70	1378.44	134.67	8.63
0.25-1 mm	410.07	278.23	154.26	104.05	1096.98	1629.24	2417.61	1629.24	149.30	9.27
free-m	408.48	367.92	267.24	107.85	1425.51	2477.77	3278.04	2477.77	158.47	10.30
P-value of ANOVA of soil properties in PF										
S	0.430	**0.009**	0.168	0.918	**0.000**	**0.000**	0.181	**0.000**	0.190	0.183
N	0.585	0.338	0.307	0.386	**0.021**	0.201	0.529	0.201	0.421	0.405

24

S × N		0.981	0.991	0.992	0.812	0.912	0.992	0.747	0.992	0.989	0.992
The difference in each soil properties before and after incubation (paired t-test)											
P-value in SF		**<0.001**		**<0.001**		**<0.001**		**<0.001**			
P-value in PF		**0.036**		**0.028**		**<0.001**		**<0.001**			

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Table 3 ANOVA results for the effect of aggregate size and N addition on 7-days cumulative GHG emissions expressed by per aggregate mass at the two forest sites (SF = secondary forest; PF = primary forest)

Source of Variations	DF	CO_2 F	*P*	DF	N_2O F	*P*	DF	CH_4 F	*P*
SF									
S	4	4.645	**0.002**	4	3.709	**0.008**	4	96.217	**0.000**
N	1	0.256	0.614	1	0.121	0.729	1	7.466	**0.008**
S × N	4	0.128	0.972	4	1.145	0.341	4	0.304	0.874
PF									
S	4	5.099	**0.002**	4	4.000	**0.007**	4	1.795	0.145
N	1	24.574	**0.000**	1	1.835	0.182	1	1.579	0.215
S × N	4	0.144	0.965	4	1.298	0.284	4	0.271	0.895

S: aggregate size, N: N addition, ×: interaction effect.

26

Table 4 GHG emission rates (calculated by per SOM) under different N additions (N0 vs. N50) and aggregate size classes (mm) at the two forest sites (SF and PF)

	SF-N0		SF-N50		PF-N0		PF-N50	
	mean	SE	mean	SE	mean	SE	mean	SE
CO_2/mg kg^{-1} h^{-1}								
4-8 mm	377.56 aA	28.63	368.41 aAB	8.58	166.51 aAB	24.41	100.53 bA	10.64
2-4 mm	349.20 aA	16.65	352.31 aAB	14.71	164.03 aAB	17.53	83.72 bA	3.06
1-2 mm	428.90 aB	13.89	383.94 aAB	11.10	154.85 aAB	4.10	93.32 bA	8.03
0.25-1 mm	433.46 aB	17.94	395.65 bA	14.39	139.84 aA	3.43	85.03 bA	9.55
Free-m	359.32 aA	14.73	350.44 aB	9.95	195.37 aB	39.26	105.48 bA	13.97
N_2O/μg kg^{-1} h^{-1}								
4-8 mm	8.96 aB	1.80	10.59 aA	2.52	7.28 aA	1.39	8.18 aA	1.55
2-4 mm	6.97 aB	0.69	7.80 aA	0.94	7.45 aA	2.08	4.19 aB	0.69
1-2 mm	8.82 aB	0.58	8.12 aA	0.54	6.76 aA	1.14	4.48 aAB	0.45
0.25-1 mm	8.64 aB	0.53	8.37 aA	0.99	5.59 aA	1.17	4.45 aAB	0.32
Free-m	13.40 aA	2.45	9.96 aA	0.64	9.24 aA	2.30	5.26 bAB	0.40
CH_4/μg kg^{-1} h^{-1}								
4-8 mm	-11.58 aC	0.93	-12.88 aC	0.67	-7.82 aA	1.62	-5.67 aA	1.14
2-4 mm	-13.41 aC	1.07	-17.38 aBC	0.99	-7.33 aA	1.58	-4.18 aA	1.64
1-2 mm	-19.69 aB	0.75	-20.26 aB	0.79	-3.75 aA	0.58	-3.99 aA	0.49
0.25-1 mm	-18.45 aB	1.14	-18.76 aB	1.46	-3.61 aA	0.88	-2.42 aA	0.81
free-m	-33.16 aA	2.40	-39.24 bA	3.48	-7.18 aA	2.63	-4.21 aA	0.44

Different lowercases indicated difference between N addition treatments in each aggregate size was determined. Different uppercases indicated difference between aggregate sizes in each N addition treatments was determined.

27

Table 5 Relationships (Pearson's correlation coefficient, r) between soil initial properties (before incubation) and GHG emission rates in each forest site (n=30)

	N_2O	CH_4	MBC	DOC	NH_4^+	NO_3^-	SOC	TN
SF								
CO_2	0.609**	-0.156	0.418*	0.482**	0.360*	0.180	0.604**	0.763**
N_2O	1	-0.375*	0.323	0.709**	0.345*	0.472**	0.651**	0.757**
CH_4		1	-0.204	-0.669**	-0.532**	-0.776**	-0.472**	-0.238
MBC			1	0.585**	0.363*	0.475**	0.182	0.332*
DOC				1	0.576**	0.738**	0.588**	0.570**
NH_4^+					1	0.579**	0.448**	0.350*
NO_3^-						1	0.471**	0.324
SOC							1	0.869**
PF								
CO_2	0.276	-0.447*	0.440*	0.313	0.168	-0.246	0.502**	0.550**
N_2O	1	-0.236	0.182	0.445*	-0.158	0.479**	-0.212	-0.126
CH_4		1	-0.327	-0.210	-0.080	0.066	0.052	-0.117
MBC			1	0.687**	0.241	-0.125	0.583**	0.686**
DOC				1	0.491**	0.302	0.348*	0.363*
NH_4^+					1	0.300	0.448**	0.426**
NO_3^-						1	-0.218	-0.220
SOC							1	0.954**

*P<0.05; **P<0.01

Figure Captions

Fig. 1 Cumulative emission of CO_2, N_2O and CH_4 as affected by aggregate sizes (4-8 mm, 2-4 mm, 1-2 mm, 0.25-1 mm and free-m) and N addition (N0 = 0 kg N ha^{-1} yr^{-1}; N50 =50 kg N ha^{-1} yr^{-1}) at the secondary forest (SF). Data was presented by mean ± standard error. The dash line presented the zero point.

Fig. 2 Cumulative emission of CO_2, N_2O and CH_4 as affected by aggregate sizes (4-8 mm, 2-4 mm, 1-2 mm, 0.25-1 mm and free-m) and N addition treatments (N0 = 0 kg N ha^{-1} yr^{-1}; N50 =50 kg N ha^{-1} yr^{-1}) at the primary forest (PF). Data was presented by mean ± standard error. The dash line presented the zero point.

Fig. 3 Global warming potential (GWP) as affected by aggregate sizes (4-8 mm, 2-4 mm, 1-2 mm, 0.25-1 mm and free-m), N addition treatments (N0 = 0 kg N ha^{-1} yr^{-1} and N50 =50 kg N ha^{-1} yr^{-1}) and forest types (secondary forest, SF and primary forest, PF). Data was presented by mean ± standard error. Different uppercases mean the significant difference between aggregate sizes was determined across N addition treatments. Different lowercases mean the significant difference between N addition treatments was determined.